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Comparison of Three Rapid Diagnostic Techniques for Detection of Respiratory Syncytial Virus from Nasal Wash Specimens

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We report results of three rapid tests for respiratory syncytial virus antigen detection. An immunofluorescence assay using commercial antibody and two commercial enzyme immunoassays (Ortho Diagnostics, Inc., Raritan, N.J., and Abbott Laboratories, North Chicago, Ill.) were applied to 199 nasal wash specimens. The Abbott enzyme immunoassay was the most sensitive technique, with a sensitivity of 93.8%. The specificities of the three techniques were comparable and greater than 95%. The availability of reliable rapid diagnostic techniques will allow for better care of infants with severe respiratory syncytial virus infection.

Respiratory syncytial virus (RSV) is a major cause of acute lower respiratory tract disease in infants and young children. Rapid and accurate diagnosis of RSV infection is important in considering antiviral treatment in severely ill patients (7, 8, 13), as well as in prevention of nosocomial infection (6, 11). Until recently, the immunofluorescence assay (IFA) has been the only reliable rapid diagnostic technique available (4). However, this technique depends upon the experience and skill of the technologist who interprets the results, as well as on the number of intact respiratory epithelial cells in the clinical specimens. A number of enzyme immunoassay (EIA) kits have become commercially available (1, 3, 9, 12), and they provide a reasonable alternative. This study compared the IFA with two commercial EIAs using viral culture as the gold standard.

Specimen collection and processing. Nasal wash specimens submitted to the clinical virology laboratory for rapid antigen detection or RSV culture or both during two epidemiologic years for RSV (1984 to 1985 and 1985 to 1986) were included in the study. The specimens were obtained by clinicians by instilling 3 to 5 ml of phosphate-buffered saline into the nares with a rubber bulb syringe and aspirating the material back into the bulb (5). The specimen was immediately transported on ice to the laboratory. Each specimen was vigorously vortexed before being centrifuged for 10 min. The cell pellet was used for IFA, the supernatant was used for culture, and the remainder was stored at −70°C until tested by EIA.

Viral isolation. A 0.1-ml portion of the supernatant was inoculated into each of duplicate cultures of primary cynomolgus monkey kidney cells, MRC-5 cells, and HEp-2 cells. The tubes were incubated at 33 and 36°C and observed for cytopathic effect daily for 10 days. Positive specimens were confirmed by IFA with RSV antiserum as described below.

IFA. The cellular pellet from the centrifuged nasal wash specimen was washed twice with phosphate-buffered saline. One drop of the cellular pellet or an inoculated tube of HEp-2 cells was spread on each of two 10-mm circles of a microscope slide. The slide was air dried and fixed in acetone for 10 min at 4°C. The cell smears were stained by bovine anti-RSV serum (Wellcome Diagnostics, Research Triangle Park, N.C.) and incubated in a humidified chamber for 30 min at 37°C. The slide was then washed and stained with fluorescein-conjugated anti-bovine antibody. After another 30-min incubation, the slide was air dried and examined for fluorescing antigen-antibody complexes. All smears contained at least a few cells per low-power field (magnification, ×100). Smears showing one or more cells stained with characteristic cytoplasmic fluorescence were considered IFA positive.

EIA. The Ortho EIA (OEIA; Ortho Diagnostics, Inc., Raritan, N.J.) was performed in accordance with the instructions of the manufacturer. In brief, the specimen and specimen treatment medium were added to murine anti-RSV-coated microtiter wells. After incubation, the wells were washed and peroxidase-conjugated bovine anti-RSV antibody was added, followed by a second incubation and washing. Substrate reagent was then added. When the third incubation was completed, the reaction was stopped and the plates were read with a spectrophotometer.

The Abbott RSV EIA (AEIA; Abbott Laboratories, North Chicago, Ill.) was performed in accordance with the instructions of the manufacturer. In brief, the specimen was added to the specimen dilution buffer and incubated with goat anti-RSV-coated beads. After an incubation, the beads were washed, rabbit anti-RSV was added, and the beads were reincubated. After the second wash, goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase was added. After another incubation, substrate solution was added and the color development was determined with the spectrophotometer provided.

A total of 199 nasal wash specimens were included in the study. Because of a limited quantity of the specimen, only 97 specimens were tested by all three rapid techniques and culture; 38 specimens were tested by two rapid techniques and culture, and 64 specimens were tested by one rapid technique and culture. Of 199 specimens, viral isolation was positive for 48. Twenty-one specimens were positive for RSV alone, and one was positive for RSV with another virus. Other specimens were positive for rhinovirus (14 specimens), influenza virus (3 specimens), cytomegalovirus (3 specimens), enterovirus (2 specimens), and adenovirus, herpes simplex virus, parainfluenza virus, and parainfluenza virus with cytomegalovirus (1 specimen each).

The IFA was performed with 167 specimens. The test was positive for 7 of 17 specimens positive for RSV by culture (sensitivity, 41.2% [Table 1]). Five specimens were positive by IFA but negative by culture; however, two of the patients

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TABLE 1. Sensitivity and specificity of three rapid tests compared with culture

Rapid test and result	No. of cultures		Sensitivity (%)	Specificity (%)
	RSV positive	RSV negative		
IFA positive	7	5		
IFA negative	10	145		
Total no. tested	17	150	41.2	96.7
OEIA positive	9	3		
OEIA negative	7	109		
Total no. tested	17[a]	113[b]	52.9	96.5
AEIA positive	15	5		
AEIA negative	1	113		
Total no. tested	16	118	93.8	95.8

[a] The results for one specimen were equivocal in the OEIA when culture was positive.

[b] The results for one specimen were equivocal in the OEIA when culture was negative.

from whom these specimens were taken had strong evidence of RSV infection. One patient had a subsequent positive culture, and the other had a brother with similar symptoms whose culture was positive for RSV.

The OEIA was performed with 130 specimens. Of 17 specimens positive by culture, 9 were positive by OEIA (sensitivity, 52.9% [Table 1]). The results for one specimen were equivocal in the OEIA when culture was positive, and the results for another specimen were equivocal when culture was negative. Three specimens were OEIA positive and culture negative (specificity, 96.5%); two of these were culture positive for enterovirus.

The AEIA was performed with 134 specimens. Of 16 specimens positive by culture, 15 were positive by AEIA (sensitivity, 93.8% [Table 1]). One specimen was negative by AEIA but positive by both culture and IFA. Five specimens negative by culture were positive by AEIA (specificity, 95.8%); one of these was culture positive for an enterovirus.

Of 97 specimens for which all three rapid tests were performed, 12 were culture positive for RSV. The results of all rapid tests and culture agreed for 81 (84%) specimens: 79 had results which were all negative for RSV (64 of these were culture negative, and 15 were culture positive for viruses other than RSV), and 2 had results which were all positive for RSV. For 16 specimens, the results of the rapid tests did not agree with those of culture; the discrepancies occurred with false-negative and false-positive rapid tests. False-negative results (10 specimens) included those for IFA alone (5 specimens), IFA and OEIA (3 specimens), OEIA alone (1 specimen), and OEIA and AEIA (1 specimen). False-positive results (6 specimens) included those for AEIA alone (3 specimens), IFA alone (1 specimen), and OEIA and AEIA (2 specimens).

We compared three widely used rapid diagnostic techniques for detection of RSV antigen in nasal wash specimens. We found that the AEIA was the most sensitive test, showing agreement with viral isolation for 128 of 134 specimens (95% agreement). The specificity of all three tests was comparable.

In our study, after vigorous vortexing, the cell pellet was removed by centrifugation for IFA use. The supernatant was used for culture and two EIAs. Removal of RSV antigen-containing cells may have reduced the sensitivity of the EIAs and culture.

The EIA for detection of RSV antigen in respiratory specimens has previously been shown to be a sensitive, accurate, and simple assay for which minimal technical skills are necessary (2, 10, 12). The latter is the major advantage of the test over the IFA. The advantages of EIA over tissue culture for identification of RSV are its relative economy, the rapidity with which the virus is identified, and the simplicity of the system. It is hopeful that management of patients with severe RSV infection will become much simpler with the reliable rapid diagnostic tests for RSV now commercially available. We recommend that viral culture be obtained simultaneously because other respiratory viruses can cause symptoms similar to those caused by RSV and these rapid tests cannot detect other respiratory viral pathogens.

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